The American Red Cross Food Ration Calculation Exercise

For food proposals it is important to calculate rations correctly. The following three exercises may help. (I.) The minimum kilocalories are the most important. II. and III. on Protein and Fat are important in terms of needs of particular populations, e.g. malnourished children, the elderly. The exercise on pages 2-3 is illustrative. The spreadsheet attachment helps you try ration rates.

I. Minimum Energy (Kilocalories)

Food rations minimum kilocalories (shortened to kcals) are calculated in four easy steps *(calculation aids are provided in italics to the right)*:

1) translate monthly food amounts from kilograms to 100-grams amounts — *Multiply the kilogram amount by 10*

2) divide the kilocalories by 30 days to come up with a daily rate — *Divide the amount in 100 grams of food by 30 (average number of days in a month)*

3) multiply the daily food intake rate by the kilocalories per 100 grams to come up with the daily kilocalorie intake per commodity [see attached Table "Approximate Nutritional Values of Commodities" for each food] — *Multiply the daily rate by the kilocalories per 100 gr.*

4) This will yield each Total per Commodity. They need to be added together for the totals per whole daily ration. — *Add the totals*

To get the % of daily minimum of 2100 kilocalories per person per day which your ration gets,

5) divide the Totals by 2100 kcals to get a % of total — *Divide the Total Minimum Energy rate by 2100 – this is the minimum ration/ person/ day, a universal standard*

Energy:

Commodity	**Amount of proposed ration**	**Amount in 100-grams of food**	**Daily rate (div by 30)**	**Kilocalories per 100 g**	**Totals**
Wheat	7 kilograms				
Rice	3 kilograms				
Lentils	3 kilograms				
Vegetable oil	2 litres (= 2 kilograms)				
Totals	15 kilograms	--	--	--	____ or ____ %

[Note: remember the standard proportions of rations are 75% grain to 15% bean to 10% oil]

Analysis: This ration is _____ (too high, too low)

Minimum Protein and Fat Levels (%)

Repeat the same steps for calculating protein and fat levels which are important in most countries, as cereals are usually the 'cheapest calories', while people often cannot afford enough protein from beans, milk, meats or enough fat from oils, butter, milk, etc.

II. Protein rations:

5) translating monthly food amounts from kilograms to 100-grams (g) amounts
6) dividing the kilocalories by 30 days to come up with a daily rate
7) multiplying the daily food intake rate by the kilocalories from the Table "Approximate Nutritional Values of Commodities" , column Protein (g) for each food to come up with the daily kilocalorie intake per commodity

Protein:

Commodity	**Amount of proposed ration**	**Amount in 100-grams of food**	**Daily rate (divide by 30)**	**Protein per 100 grams**	**Totals**
Wheat	7 kilograms				
Rice	3 kilograms				
Lentils	3 kilograms				
Vegetable oil	2 litres (= 2 kilograms)				
Totals	15 kilograms	--	--	--	

Note: remember the accepted ranges of protein intake is 40-60 grams (10-12% of energy intake) and fat intake is 40-45 grams (17-20% of energy intake)

III. Fat Rations:

8) translating monthly food amounts from kilograms to 100-grams (g) amounts
9) dividing the kilocalories by 30 days to come up with a daily rate
10) multiplying the daily food intake rate by the kilocalories from the Table "Approximate Nutritional Values of Commodities" , column Fat (g) for each food to come up with the daily kilocalorie intake per commodity

Fat:

Commodity	**Amount of proposed ration**	**Amount in 100-grams of food**	**Daily rate (divide by 30)**	**Fat per 100 grams**	**Totals**
Wheat	7 kilograms				
Rice	3 kilograms				
Lentils	3 kilograms				
Vegetable oil	2 litres (= 2 kilograms)				
Totals	15 kilograms	--	--	--	

Note: remember the accepted ranges of protein intake is 40-60 grams (10-12% of energy intake) and fat intake is 40-45 grams (17-20% of energy intake)

NOTE: THIS EXEMPLE ILLUSTRATES INDIVIDUAL RATION SIZES; they are not for households; please take the figures and *multiply* by the number of individual rations needed for the household ration, e.g. x 5 for a family of 5.

IF YOUR RATION IS ALREADY SET FOR HOUSEHOLDS, then *divide* the ration by the average household size to get to the daily individual rate, e.g. ./. 5 for a family of five. The difficulty comes in when households have many children who, by definition, need only a proportion of a full adult ration. You may want to adjust the ration proportionally if you know their ages. (see table, below). Yet given intra-household sharing, it still may be legitimate to work off of 2100.

This American Red Cross document was downloaded from the Food Aid Management (FAM) web site: http://www.foodaid.org.

The USAID Commodities Reference Guide (www.usaid.gov/hum_response/crg) suggests the following:

Table 1: 100% Recommended Daily Allowances for Different Age/Sex groups*

Age/Sex	Recommended Energy Allowance (kcal/d)	Recommended Protein Allowance (g/d)	Fat (g/d)
Children 1-3 yrs	1300	16	45-58
Children 4-6 yrs	1800	24	40
Children 7-10 yrs	2000	28	45
Non-pregnant female 11-50 yrs	2200	47*	45-50
Female 51+ yrs	1900	50	36-42
Male 11-14 yrs	2500	45	50-56
Male 15-18 years	3000	59	57-67
Males 19-50 years	2900	60	55-65
Males 51+ years	1900	63	36-42
Pregnant female 20+ years[2]	+300	+13	+6-7
Lactating female 1st 6 mos	+500	+18	+10-11
Lactating female 2nd 6 mos	+500	+15	+10-11

*From the National Research Council's Recommended Dietary Allowances. National Academy Press, 1989.

The Commodities Reference Guide not only has programming guidance re: various sectors such as Safety Nets, Maternal Child Health, and Food For Work, but also has nutritional and transport information on the commodities, below:

Food Commodity Fact Sheets

1. Beans, Black	2. Corn Masa Flour
3. Beans, Blackeye (Cowpeas)	4. Corn-Soy Masa Flour, Instant
5. Beans, Great Northern	6. Lentils
7. Beans, Kidney (Light Red, Dark Red, All types)	8. Peas
9. Beans, Navy (Pea Beans)	10. Rice
11. Beans, Pink	12. Rice (Parboiled)
13. Beans, Pinto	14. Sorghum
15. Beans, Small Red	16. Sorghum Grits, Soy-Fortified (SFSG)
17. Bulgur (BW)	18. Soybeans
19. Bulgur, Soy Fortified (SFBW)	20. Soybean Meal
21. Corn (bagged, bulk)	22. Fortified Refined Vegetable Oil
23. Cornmeal	24. Wheat
25. Cornmeal, Soy-Fortified (CMSF)	26. Wheat Flour
27. Corn Soy Blend (CSB)	28. Wheat Soy Blend (WSB)

Food Ration Calculation Exercise Answers (example = Armenia)

Energy:

KILOCALORIES (kcals)

Commodity	Proposed ration (kg)	Amount in 100-grams of food	Daily rate (div by 30)	Kilocalories per 100 g	Totals
Wheat flour	7.00	70.00	2.33	350.00	816.67
Rice	3.00	30.00	1.00	330.00	330.00
Lentils	3.00	30.00	1.00	335.00	335.00
Vegetable oil	2.00	20.00	0.67	885.00	590.00
Totals	15.00	--	--	--	2071.67
Percent of daily minimum (2100 kcals)					99%

[Note: remember the standard proportions of rations are 75% grain to 15% bean to 10% oil]

Analysis: This ration is fine. The kcals calculation is always the over riding one, as most protein and fat will be gotten through this energy. The ration mix + prioritization was chosen through discussions with communities + Armenian RC.

Protein:

PROTEIN (%)

Commodity	Amount of proposed ration	Amount in 100-grams of food	Daily rate (divide by 30)	Protein per 100 grams	Totals
Wheat flour	7	70.00	2.33	11.5	26.83
Rice	3	30.00	1.00	7	7.00
Lentils	3	30.00	1.00	22	22.00
Vegetable oil	2	20.00	0.67	0	0.00
Totals	15				55.83

Note: remember the accepted ranges of protein intake is 40-60 grams (10-12% of energy intake)

Analysis: This ration, 55.8 grams of fat is high but within the normal range. Given the low diet diversity in Armenia and the very low protein intake, particularly in urban areas, this is needed. Generally, as most populations are protein-deficit, the more the protein the better.

Fat:

FAT (%)

Commodity	Amount of proposed ration	Amount in 100-grams of food	Daily rate (divide by 30)	Fat per 100 grams	Totals
Wheat flour	7	70.00	2.33	1.5	3.5
Rice	3	30.00	1.00	0.5	0.50
Lentils	3	30.00	1.00	1.5	1.50
Vegetable oil	2	20.00	0.67	100	66.67
Totals	15				72.17

Note: remember the accepted ranges of fat intake is 40-45 grams (17-20% of energy intake)

Analysis: This ration, 72.2 grams of fat is almost double the normal range which can be a problem, especially hypertensive older people. Yet oil is an important weaning food for children age 2 and under (see above, who are largely the recipients in urban Armenia) and given the high-cost of oil, it might be more beneficial than harmful.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Jacques-Philippe Moulinoux et al.	Confirmation No.: 7755
Appln. No.	:	10/566,785	Group Art Unit: 1611
Filed	:	January 12, 2007	
For	:	USE OF A POLYAMINE-POOR COMPOSITION FOR THE PRODUCTION OF A MEDICAL HUMAN FOOD	Examiner: Klinkel, Kourtney L.
Docket No.	:	U16.12-0006	

DECLARATION UNDER 37 C.F.R. § 1.132

VIA ELECTRONIC FILING
JUNE 7, 2010

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Guy Simonnet, hereby declare as follows:

1. I am a co-inventor of the subject matter of the above-identified patent application.
2. I declare that from 1975 until present I have been employed as a Professor at the University Victor Ségalen - Bordeaux 2, Bordeaux, France
3. I declare that I have a degree in PhD in Pharmacy from the University Victor Ségalen - Bordeaux 2, Bordeaux, France (1968)
4. I declare that I have been employed as a Professor in the field of Cell biology and Neuropharmacology for 25 years
5. I declare that the attached document as Exhibit A describes the current understanding in the field of pain relief.
6. I declare that the experiments described and discussed in Exhibit A are accurate.
9. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these

statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

Inventor: Guy Simonnet
(Printed Name)

Inventor: _____
(Signature)

Date: June 4, 2010

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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EXHIBIT A

of

DECLARATION UNDER 37 C.F.R. § 1.132

ANNEX

Background

The claim for an "**anti-hyperalgesic effect**" for Polyamine deficient compositions is different from the previous claim "**analgesic effect**" as indicated in 93 14761 (N° Publication 2 706 255).

According to a modern view of relieving pain, it is necessary to make a difference between:

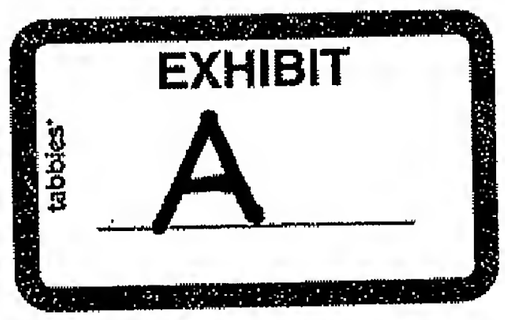
- **Analgesic effect** which is defined by an acute decrease of pain sensation related to a tissue injury (see Fig.1, effect 1).
- **Anti-hyperalgesic effect** which is the specific reduction of pain sensitization process (pain facilitatory systems) which induce exaggerated pain sensation (hyperalgesia) in response to a given nociceptive stimulus (see Fig.1, effect 2) or abnormal pain sensation in response to a non nociceptive stimulus (allodynia).

Indeed, these two therapeutic effects are related to two different neurophysiologic processes: the first one (analgesia)is related to the reduction of the activity of nociceptive pathways, and the second one (anti-hyperalgesia) is related to the inhibition of the overactivation of specific "facilitatory systems", which are **different from nociceptive pathways,** and lead to pain hypersensitivity (exaggerated pain sensation).

It is noteworthy that it is now well admitted that these last systems, *i.e.*, pain facilitatory systems, play a critical role in the development of chronic pain.

These differences between analagesia and anti-hyperalgesia were not known in the 90's when the first patent 93 14761 (N° Publication 2 706 255) was filed. Therefore, **specific experimental models (as clinical approaches) to differentiate hyperalgesia from analgesia were not developed in 1993,** leading to the impossibility to distinguish and to claim anti-hyperalgesia from analgesia and therefore to claim for an anti-hyperalgesic effect of PDD (Polyamine Defficient Diet, according to the invention) in 90's.

(see definitions from "International Association for the Study of Pain", IASP).



Pain definitions
(According Internal Association for Study of Pain)

Pain

An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.

Analgesia

Absence of pain in response to stimulation which would normally be painful.

Hyperalgesia

An increased response to a stimulus which is normally painful.

Note: *Hyperalgesia reflects increased pain on suprathreshold stimulation. For pain evoked by stimuli that usually are not painful, the term allodynia is preferred, while hyperalgesia is more appropriately used for cases with an increased response at a normal threshold, or at an increased threshold, e.g., in patients with neuropathy. may develop as a result of long-term opioid use in the treatment of chronic pain.Various studies of humans and animals have demonstrated that primary or secondary hyperalgesia can develop in response to both chronic and acute exposure to opioids. This side effect can be severe enough to warrant discontinuation of opioid treatment.*

Allodynia

Pain due to a stimulus which does not normally provoke pain.

Note: *The term allodynia was originally introduced to separate from hyperalgesia and hyperesthesia, the conditions seen in patients with lesions of the nervous system where touch, light pressure, or moderate cold or warmth evoke pain when applied to apparently normal skin.*

From analgesia to anti-hyperalgesia : differences and specific mechanisms

According to a modern view point recently developed in 1990-2000 years (see Fig.1), clinical pain, especially chronic pain, **is not a only a reflection of sustained noxious input** and it is, to a large extent, the expression of changes produced in the central nervous system (CNS), including the phenomenon of **central sensitization** that by increasing gain, elicits **hyperalgesia/allodynia** (see Fig.1) **and is a critical component of chronic pain** (C.J. Woolf and M.W. Salter, Science, 288: 1765-1768, 2000; J. Scholz and C.J. Woolf, Nature Neurosci. 5 Suppl: 1062-1067, 2002).

As suggested by numerous authors, it is necessary to treat both the disease/injury process in the periphery (see Fig.1: strategy 1: analgesia), and also the changes it induces or triggers in the CNS leading to pain sensitization, (see Fig.1: strategy 2: anti-hyperalgesia).

Prevention or reduction of central sensitization will substantially eliminate the hyperalgesia and allodynia that patients find so distressing (see Fig.1: strategy 2: anti-hyperalgesia), and it offers **new possibilities for the development of new therapeutic strategies for relieving pain** (Fig.1).

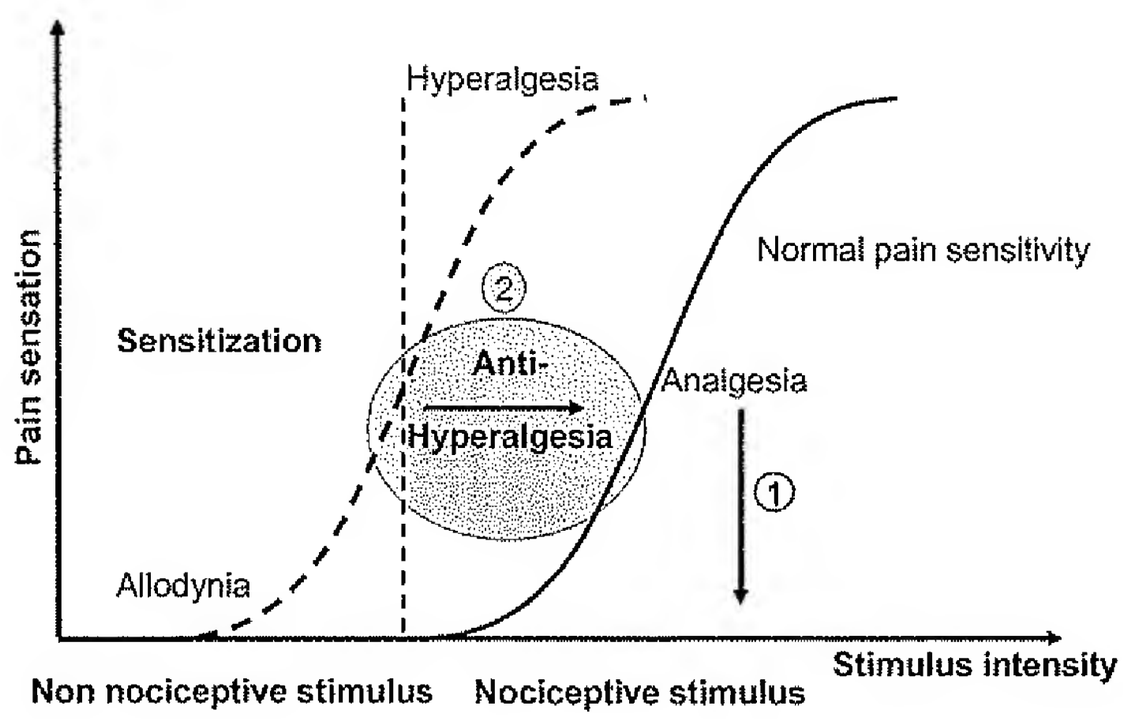


Fig. 1. Relation between nociceptive stimulus intensity and pain sensation level indicating the new concept of « anti-hyperalgesia strategy» which is different from « analgesia strategy ».

Based on these new pain concepts, an anti-pain hypersensitivity or anti-hypearlgesic strategy (Fig.2) does not require to induce analgesia on acute pain via a direct action on pain pathways in the CNS but has **to induce blockade or negative modulation of neural networks specifically involved in pain sensitization processes (pain facilitatory systems). Interestingly, these sensitization processes are supported by neural systems different from pain pathways** (C.J. Woolf and M.W. Salter, Science, 288: 1765-1768, 2000; J. Scholz and C.J.

Woolf, Nature Neurosci. 5 Suppl: 1062-1067, 2002). Therefore, an innovative and fruitful strategy for relieving abnormal pain as exaggerated postoperative pain or chronic pain, is **to prevent or to reduce the development of central sensitization by specific anti-hypersensitivity strategies as a complementary strategy to classical analgesic drugs (balanced analgesia).**

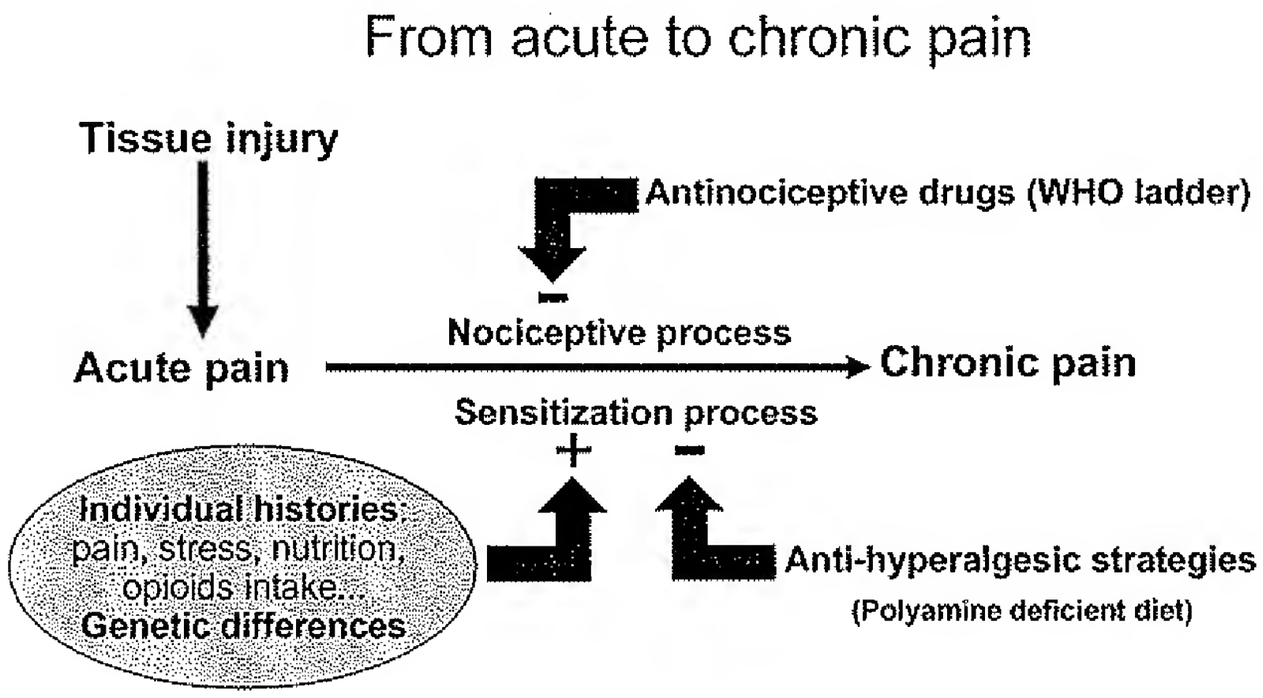


Fig. 2. Model of chronic pain process indicating the critical role of sensitization process and proposals of new therapies as Polyamine deficient diet (PDD)

In other words, this leads to the **new concept** that it is possible to produce analgesia by specific inhibition of pain hypersensitivity (pain facilitatory systems) in patients with abnormal pain as it is observed in exaggerated postoperative pain and in some forms of chronic pain despite the fact that anti-pain hypersensitivity strategy has no direct action of nociceptive pathways as it is the case for classical analgesic drugs.

As indicated in Fig.1 and Fig.2, a modern pain relief strategy has:

i) to reduce pain (**analgesic effect**), via inhibition of nociceptive pathways, by classical analgesic drugs as proposed by the World Health Organisation ladder (3 steps WHO ladder) and - our claim 93 14761 (N° Publication 2 706 255)

and

ii) to reduce pain by preventing and/or reducing pain hypersensitivity (**anti-hyperalgesic effect**), via specific inhibition of pain facilitatory systems which amplify pain sensation (gain system) and lead to exaggerated pain following a tissue injury

Nowadays, no specific anti-pain hypersensitivity has been developed in the pharmacologic field and **"Polyamine deficient diet" is the first therapeutic strategy specifically acting on pain sensitization process without side effects.**

Preclinical results and comments

As shown in the new figure (Fig. 3, *not shown inpresent*), PDD has no effect on basal nociceptive threshold (NT) in healthy animals suggesting that PDD do not directly affect the level of pain sensitivity (analgesic effect) via an inhibition on nociceptive pathways as produced by classical analgesics according WHO ladder.

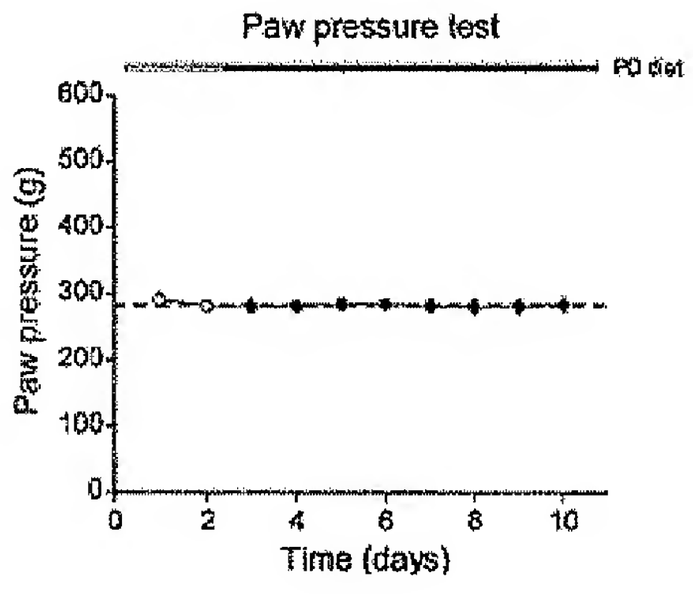


Fig. 3. PD diet does not induce any changes on basal pain sensitivity in non painful rats, indicating that PD diet has no analgesic effect *pers se*. The mechanic nocieceptive threshold is measured as the rat vocalization in response to the paw pressure (g) of an hindpaw.

As previously shown in **Fig.** 1/4 of the present application, the new Figure 4 confirms that rats fed with PDD for 7 days before a tissue injury (inflammation performed in a rat hind paw, on D_0) show a reduction of post-inflammatory hyperalgesia observed for several days following a first inflammation (D_0-D5). This PDD inhibitory effect on post-inflammatory hyperalgesia is more pronounced after a new inflammation performed 7 days later suggesting that PDD has prevented the physiological enhancement of pain sensitivity in animals with a prior pain history (first inflammation). Fig.4 (hyperalgesic index, defined as the area under the curve) shows that PDD has no acute analgesic effect (D_0) but totally suppress the hyperalgesic index enhancement, observed following the second inflammation.

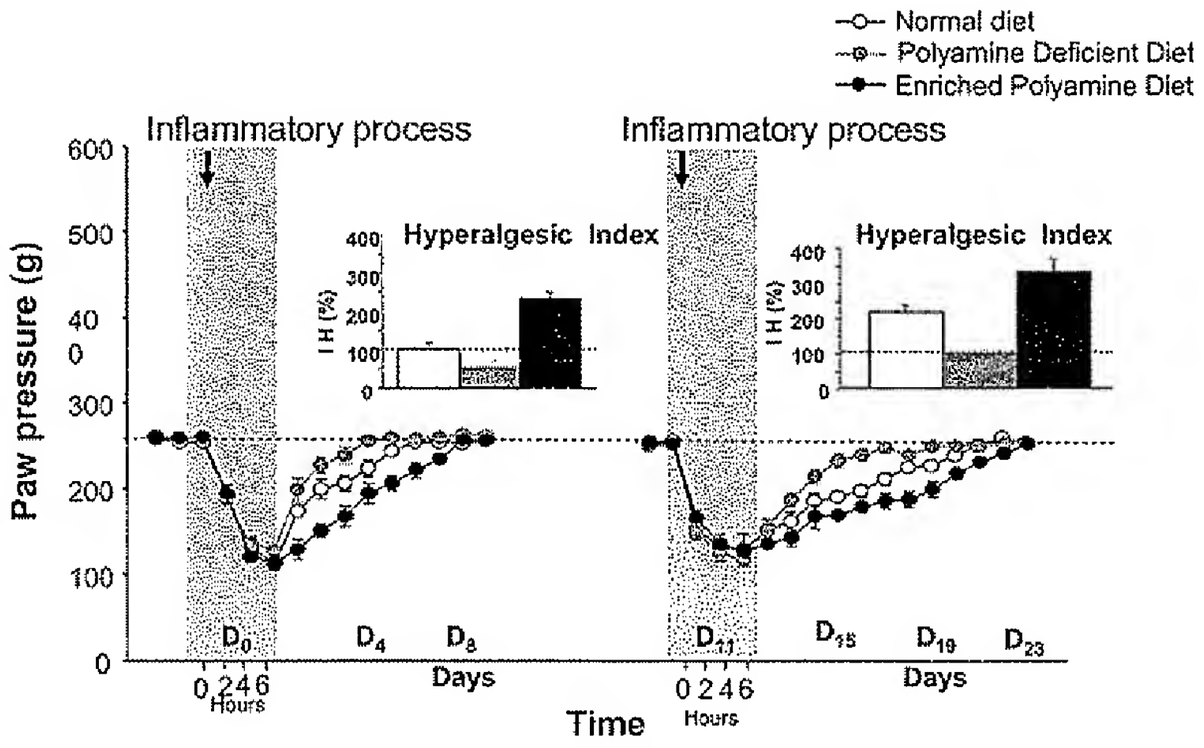


Fig. 4. Repetition of inflammation in a rat hindpaw induced an enhancement of pain sensation (hyperalgesia). Polyamine deficient diet reduces hyperalgesia and Polyamine enriched diet (10 times) increase hyperigesia as measured by exaggerated decreases of mechanical nociceptive threshold (vocalisation in response to hindpaw pressure (g))

Interestingly, new data in new Fig. 4 show **that in contrast to a polyamine deficient diet, a Polyamine enriched diet** (10 times enriched as compared to normal chow for rats) **strongly enhances the hyperalgesia** observed during the following days after both the first and the second inflammations, confirming that **polyamines play a critical role in the development of pain sensitization processes leading to hyperalgesia.**

Moreover, it has been previously demonstrated that opioids induce hyperalgesia (OIH) following analgesia and enhances hyperalgesia following a tissue injury (surgical incision or inflammation) leading to exaggerated postoperative pain in animals (Celerier et al. Anesthesiology, 92: 465-472, 2000, Rivat et al. Anesthesiology, 96: 381-391, 2002, Richebe et al. Anesthesiology 102: 421-428, 2005) and humans (Angst et Clark Anesthesiology 104:570-587, 2006). Interestingly, **PDD reduces the fentanyl pain enhancement** (fentanyl is an opioid largely used for surgery in humans) observed in rats after inflammation or surgical incision (see Fig. 2/4 of the present application) despite the fact that PDD does not induce any acute analgesic effect (no reduction of the nociceptive threshold decrease observed on D_0, the inflammation day).

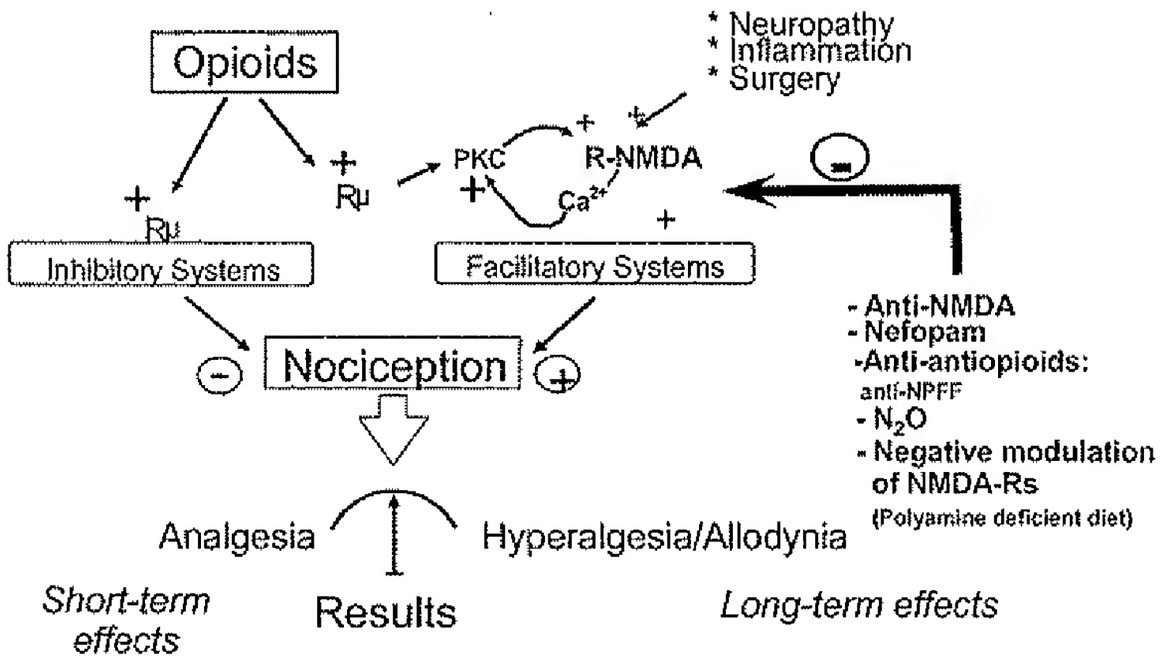


Figure 5. Neurobiological model showing that NMDA receptor systems play a critical role in pain sensitisation process induced by both nociceptive inputs and opioids. According this model, pain is the result of a blance beteween pain inhibitory and pain facilitatory systems.
(ref: Simonnet and Rivat Neuroreport 14:1-7, 2003)

Interestingly, Fig. 6 shows that the dramatic enhancement of inflammatory hyperalgesia induced by a series of prior non nociceptive environmental stress or NNES (rats were placed for one hour in a new experimental room, in new boxes with fresh litter, and were exposed to a light - 350 lux - placed 2 m away from the rat boxes). This **exaggerated inflammatory hyperalgesia induced by prior NNES was totally suppressed in rats fed with PDD.**

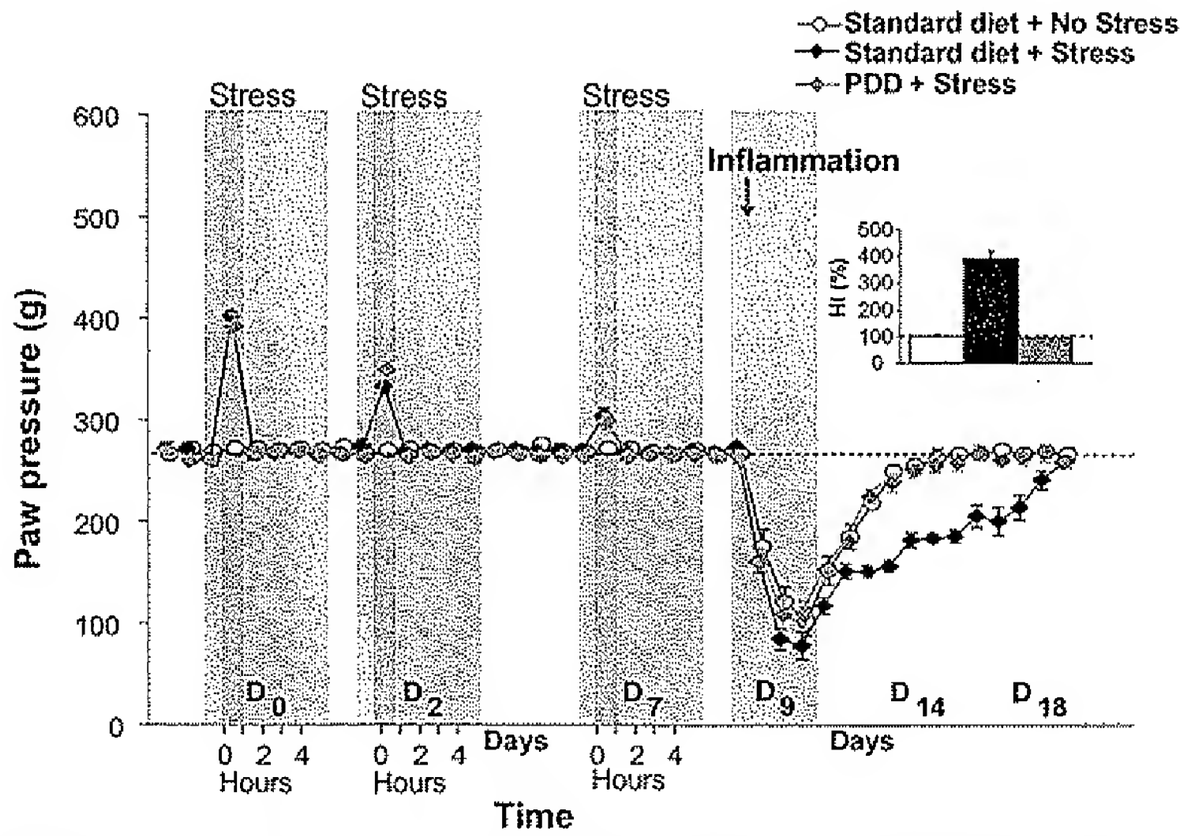


Figure 6. Preventive effect of PDD on enhancement of inflammatory hyperalgesia by prior non-nociceptive environmental stress

These data clearly confirm that **PPD does not act mainly as a classical analgesic by an acute reduction of pain, but is acting by specifically reducing pain hypersensitivity leading to an anti-hyperalgesic effect.**

This claim is also supported by results at cellular level at spinal cord level showing that PDD does not affect nociceptive pathway functioning but selectively reduces overactivation of cellular mechanisms involved in pain sensitization process.

Numerous studies indicate that changes in synaptic excitability play a critical role in the development of central sensitization leading to pain hypersensitivity in exaggerated postoperative pain (M.S. Angst et J.D. Clark, Anesthesiology 104:570-587, 2006, O.H. Wilder-Smith et al. Anesthesiology 104: 601-607, 2006) and in chronic pain (H. Kehlet and J.C.Woolf The Lancet 367: 1618-1625, 2006). It is well admitted that overactivation of glutamate/NMDA receptors (Fig. 5) play a critical role in this activity-dependent central sensitization process leading to hyperalgesia and allodynia (C.J. Woolf and M.W. Salter, Science, 288: 1765-1768, 2000, Simonnet et Rivat Neuroreport, 14:1-7, 2003, Guo et al. J. Neurosci. 24: 9161-9173, 2004).

Therefore, though antagonists of NMDA receptors (NMDARs) have not acute analgesic efefct *per se* (as PDD) at clinical doses, a single administration of an NMDAR antagonist as ketamine prevents the development of pain hypersensitivity in animal models (Richebe et al. Anesthesiology 102: 421-428, 2005) as it prevents the development of exaggerated postoperative hyperalgesia in humans (Guignard et al. Anesthesiology 93: 409-417, 2000), Angst et Clark Anesthesiology 104:570-587, 2006). They also reduce some chronic pain syndromes which are resistant to classical analgesic drugs (Chizh et al. Trends Pharmacol Sci 12:636-642 2005) indicating the critical role of these neural systems in hyperalgesia and chronic pain. Unfortunately, NMDAR antagonists, are generally poorly tolerated and can not be used in humans, especially over long-lasting use, because they induce unacceptable effects at therapeutic doses.

It is the advantage of PDD to be a safe anti-hyperalgesia strategy (without noticeable side effects for several weeks use) as compared to the use, especially the chronic use, of NMDAR anatagonists in animals (Rivat et al. Pain 137: 125-137, 2007) and humans (Cipolla et al. Amino acids 33:203-212; 2007; Cipolla et al. Biomed Pharmacother. 2010, in press).

Cellular studies on spinal cord tissue indicate Fig 3/4 of the present application that PDD for 7 days does not affect the basal level of tyrosine phosphorylation of the spinal NR2B sub-unit-containing NMDAR but prevents its enhancement associated with inflammatory hyperalgesia. This confirms that **the main effect PDD is to prevent pain hypersensitivity by negatively modulating the overactivation of NMDAR associated with hyperalgesia.**

These results lead us to evaluate the ability of PDD to prevent or to reduce tolerance to analgesic effects of opioids.

Complementary experimentations

In a **first complementary experimentation**, a group of rats was fed with a standard diet, whereas another group of rats was fed with PDD.

The two groups were treated with a daily subcutaneous administration of 0.3mg/kg of heroin during 14 days (D1 to D14). This administration was stopped until the 14th day and restarded the 28th day, and was stopped again. From the 14th day the group of rats fed with PDD was fed like the other group of rat with standard diet.

Daily, the nociceptive level of the rats was measured through the classical test of pressure on the leg and measurement of the emitted screams (modified Randall and Selitto test).

Fig. 7 shows the evolution in time of the daily measured nociceptive levels in the two groups of rats. This figure shows that the repeated administration of heroin leads to a progressive decrease of nociceptive level (the acute heroin analgesic effect is not shown), i.e. a hypersensitivity to pain in the group of rats having a standard diet.

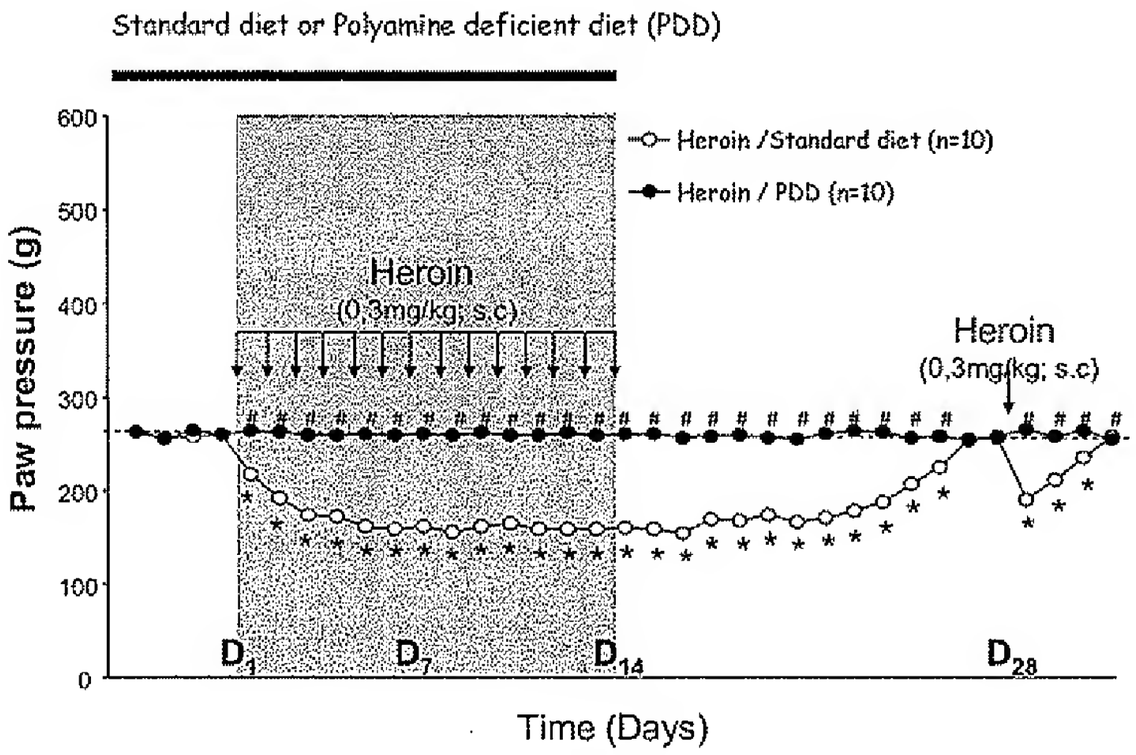


Fig. 7 Preventive effect of PDD on hyperalgesia induced by once daily heroin administration. Nociceptive mechanical threshold (vocalisation in response to paw pressure on a hind paw) is dertermined just before each daily heroin administration

*Test de Dunnet # Test de Newman Keuls

Fig. 7 also shows that the administration of a dose of 0.3mg/kg of heroin, once all the animals have reached again a basis nociceptive level after a weaning of heroin since D14, induces a 3 days hyperalgesia for the rats fed with a standard diet (an immediate analgesia is of course observed after injection as showed in D28 - part of Fig. 8). This hyperalgesia is fully suppressed for rats fed with PDD until weaning, despites this diet was stopped for 14 days and replaced by standard diet.

Curves and histograms presented in Fig. 8 give a more precise comparison of analgesic effects of heroin in the hours following its administration on rats of the two groups the 1st, 7th and 14th days (D1, D7, D14) of the period during which the heroin was administered, and the 28th day after the administration was stopped and restarted.

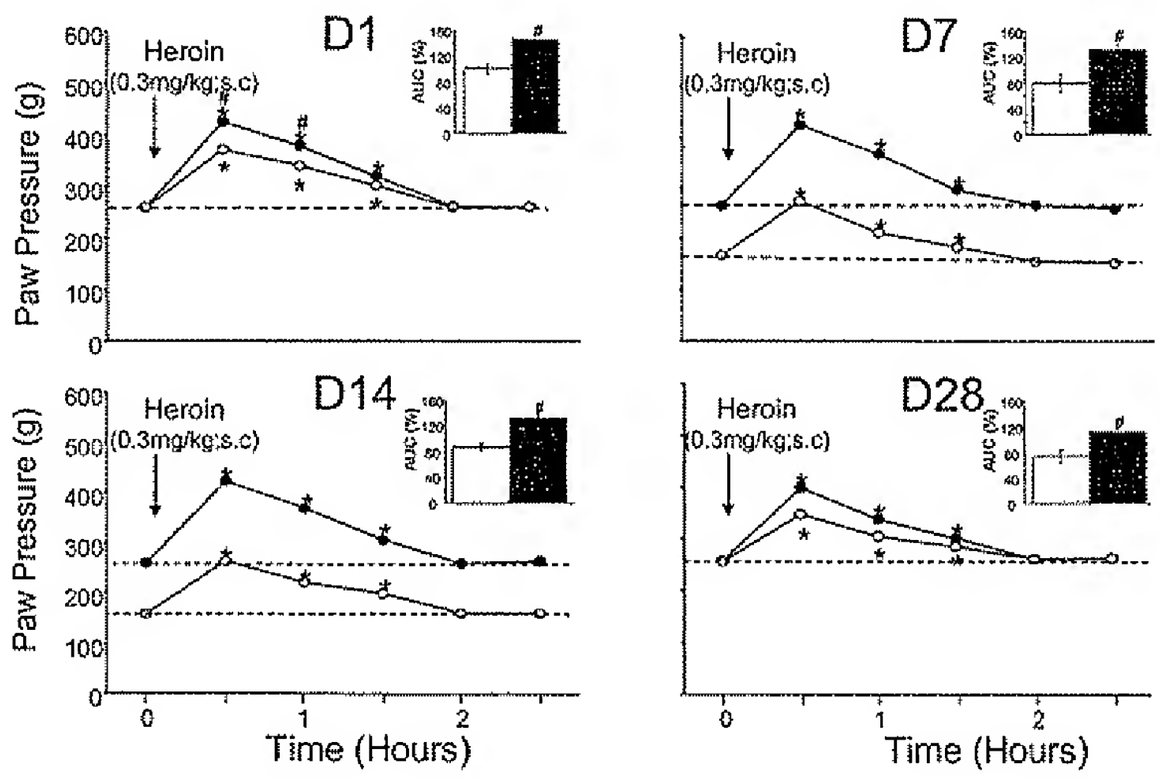


Fig. 8 Comparative analgesic effect of 0.3mg of heroin on before (D_0), during (D_7, D_{14}) and after the cesssation of a series of once daily heroin administration (0.3mg/kg) according the experimental design described in Fig.7 (dark lines: PDD group)

The curves of these figures show that the rats fed with a standard diet have a progressive tolerance for heroin, the maximum effect of this compound decreasing sharply, whereas rats fed with PDD have no tolerance for this compound. In addition the histograms associated to these curves also show that the area under curves (AUC) stay constant in every group of rat showing that the pharmaceutical analgesic effect of heroin remains the same.

These results show that the decrease of the maximum effect of heroin, i.e. the apparition of tolerance for this compound is essentially due to the development of an hypersensitivity for pain traduce by a decrease of nociceptive level.

The two histograms of Fig. 9 show in addition that the same pharmacologic analgesic is enhanced thanks to PDD.

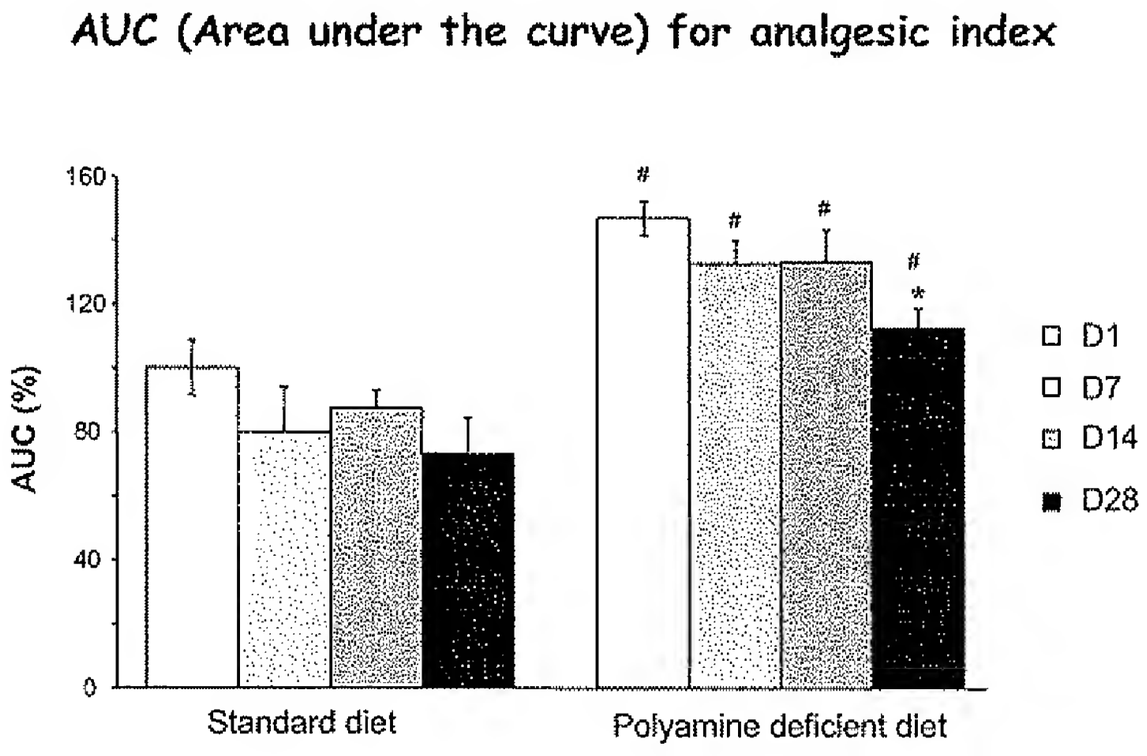


Fig.9 Analgesic index for 0.3 mg/kg heroin as described in Fig. 8

The results of this first experimentation clearly show that the invention enables to prevent fully and with efficiency the apparition of hypersensitivity to pain involved by repeated administrations of heroin and that a PDD can be used for the implementation of a therapeutically efficient food designated to combat tolerance to analgesic effects of opioid analgesics.

In a **second complementary experimentation** four groups of rats were fed with a standard diet. For one of the group of rats, the standard alimentation was replaced after 14 days (at the end of heroin administration period) and during 14 days by a PDD.

Two groups of rats fed with standard diet were submitted to a daily subcutaneous administration of 0.3mg/kg of heroin during 14 days (D1 to 14). This administration was stopped during 14 days (until 27th day) and restarted after 28th day (D28) and stopped again. As a reference, another group of rats (control group) had no administration of product and another one had an administration of a saline solution (saline group).

The curve of Fig. 10 shows that rats fed with PDD go back very fast to a basis nociceptive level after weaning of heroin (D14). In the opposite rats fed with standard diet take 13 days to go back to this basis level.

Fig.10 also shows that rats fed with PDD do not show a 3 days hyperalgesia after the new injection of heroin and do not show hyperalgesia due to non-nociceptive environmental stress (NNES). NNES (change of cage and sawdust during one hour associated to an augmentation of light) was applied to all groups of rats after they were back to basis nociceptive level.

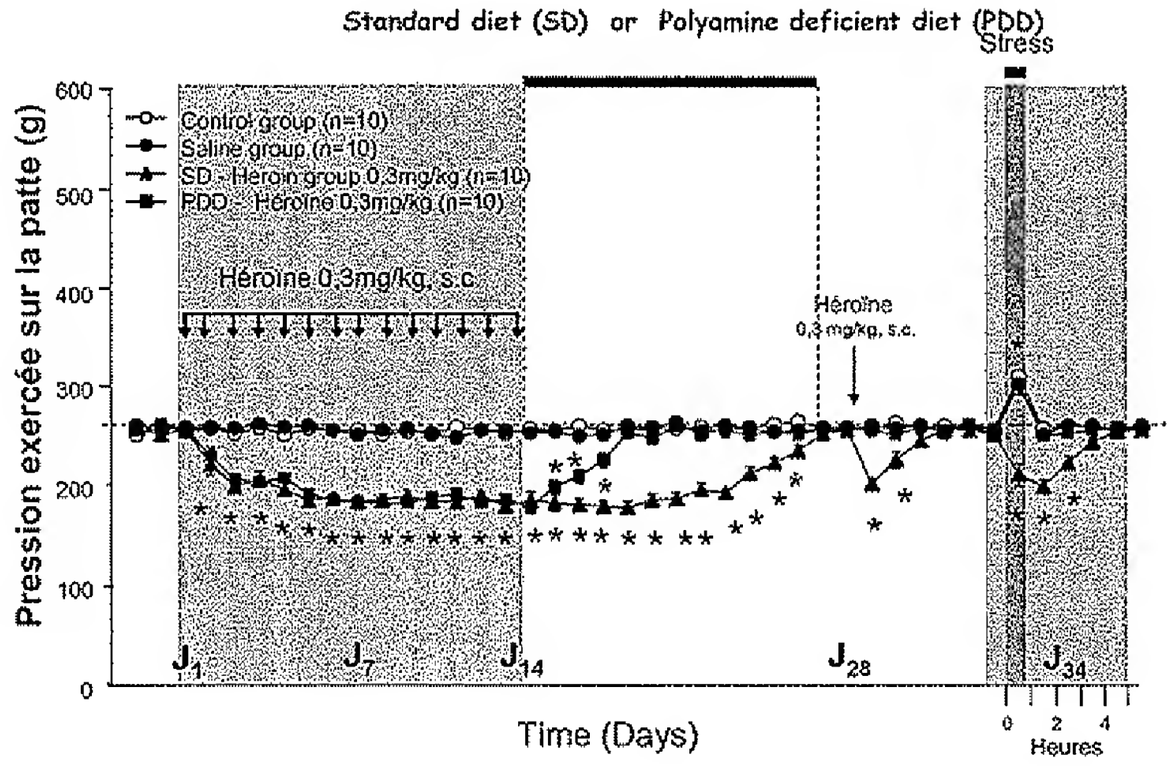


Fig. 10. PDD reduces hyperalgesia associated with the heroin withdrawal syndrom following a series of once daily heroin administration for 2 weeks
*Test de Dunnet # Test de Newmans Keuls

Figure 11 indicates that PDD strongly reduces the anxiety-like behaviour observed during the withdrawal heroin syndrom in rats once-daily treated by 0.3mg of heroin for 2 weeks according the experimental design described for Fig. 10. Anxiety-like behaviour is measured by using the classical Elevated Plus-Maze test which compare the number of entry in open arms versus enclosed arms of the apparatus. The anxiety-like behaviour test was performed 5 days after the end of heroin administration (D19) in rats fed with standard diet or fed with PDD from the beginning of heroin withdrawal (D14).

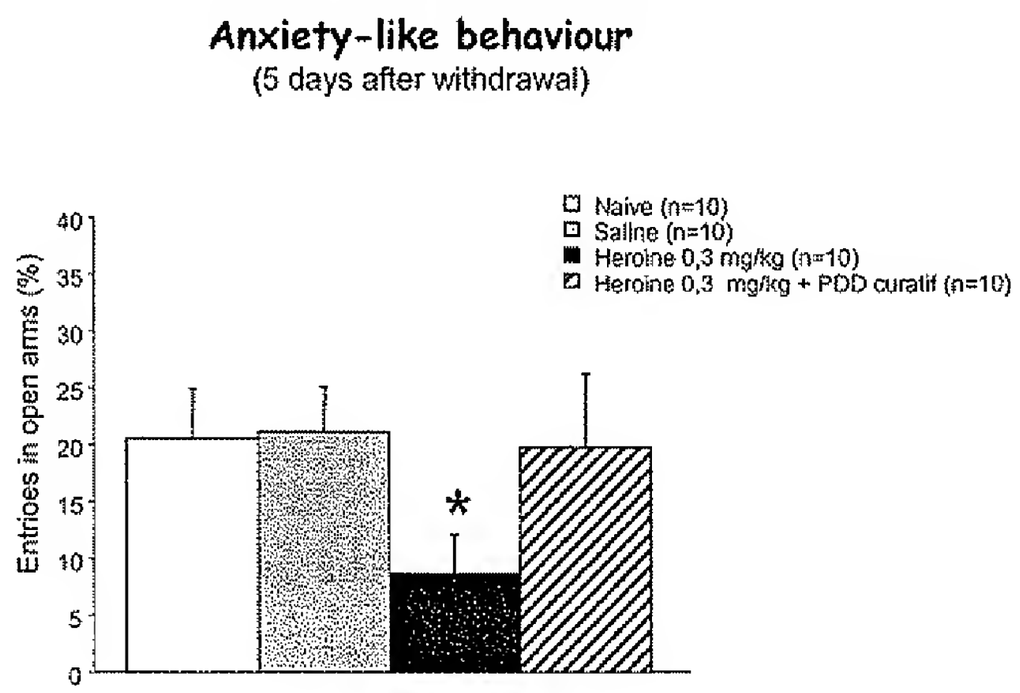


Fig. 11. Reduction of anxiety-like behaviour associated with the heroin withdrawal syndrom in rats fed with PDD from the beginning of the heroin withdrawal syndrom (see experimental design in Fig.10)

Document figure 12 indicates that PDD strongly reduces the anxiety-like behaviour observed in rats with prior pain and opioid experiences.

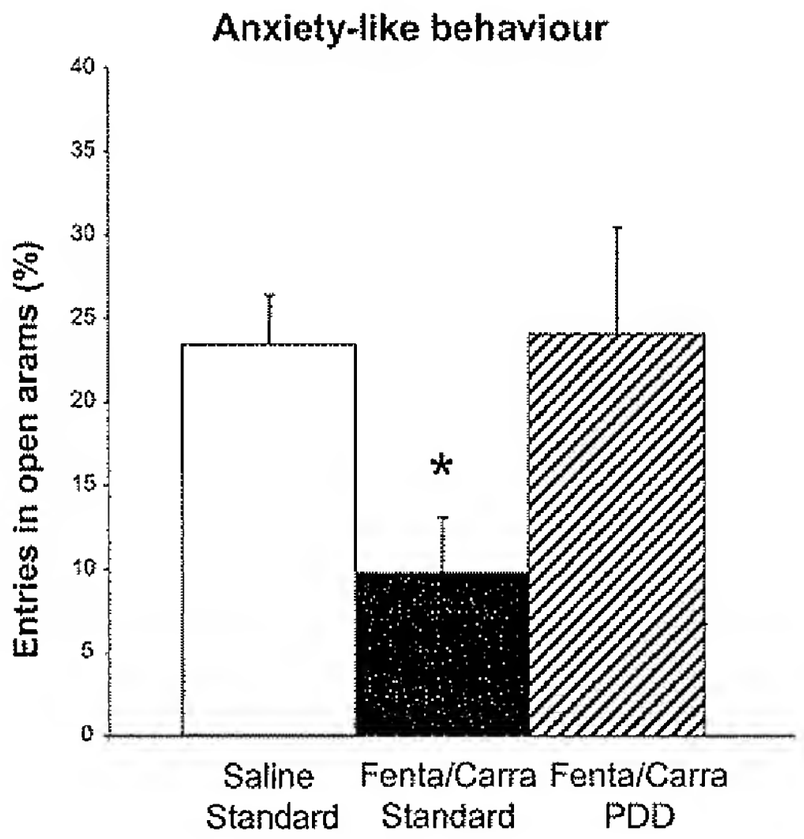


Fig.12. Reduction of anxiety-like behaviour by PDD in rats with pain and opioid experiences. The Elevated-Plus maze was performed in rats fed with nporma! chow or PDD,2 weeks after the tissue injury assocaited with an opioid (fentanyl) administration

The results of this second experimentation show the curative effect on hypersensitivity to pain due to repeated administration of heroin or linked to the use of endogenous opioids by (non-nociceptive) environmental stress observed thanks to the invention.

Comparison between patent n° 2 706 255 and the present application

In patent n° 2 706 255, in example 2, the analgesic effect is tested: the experience is a comparison between two groups of rats which are treated during 7 days with PDD or standard diet. The test is "tail flick test", where the resistance to pain is instantly measured. The analgesic effect of the treatment is tested: the measurement is made on the instantaneous effect of an instantaneous noxious stimulus.

In the present application, the memory of the pain is measured through the weaning of heroin and the restarts of injections of heroin: the experiment was carried out on the anti-hyperalgesic effect of the treatment.

Patent n°2 706 255 could not suggest the anti-hyperalgesic effect of the treatment because the test did not study the evolution of pain on the time span but just the instantaneous response to a stimulus. Furthermore in patent n°2 706 255 the conditions of the experiment could not enable the existence of the anti-hyperalgesic effect and a fortiori said effect could not be determined.